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A MODIFIED SLIDE CULTURE TECHNIQUE FOR QUICK DRUG SENSITIVITY TESTING OFM *TUBERCULOSIS**

P.R. Gupta, B. Singhal, T.N. **Sharma and R.B. Gupta**

Summary. For one hundred sputum specimens from untreated smear positive patients of pulmonary tuberculosis slide cultures were put up on drug free and drug containing selective blood culture media. The slides were examined for micro colonies under bright field microscope using ZN technique after seven days of culture. Simultaneously, the sputa were examined by indirect culture and sensitivity testing for comparison.

The modified slide culture technique was found to be a safe, rapid and effective alternative to indirect sensitivity test.

Introduction

The results of a study done in Hong Kong have revealed that pretreatment drug sensitivity pattern of *M. tuberculosis* does not influence the outcome of chemotherapy in untreated cases of Pulmonary tuberculosis[1]. However, in treatment failure patients, retreatment based solely on the history of previous treatment may not be effective and result in inadvertent loss of a drug that might otherwise have been necessary. Indirect sensitivity test results become available only after 10 to 12 weeks and a patients should not wait that long to start treatment. The advantage of having an efficient and rapid culture and sensitivity testing becomes obvious in such a situation.

Radiometric methods have been developed for rapid sensitivity testing[2,3]. Though effective, these techniques are costly. Several slide culture techniques were introduced to fulfil the need[4,5,6,7] but were discarded later as these were too cumbersome and even potentially dangerous.

Dickinson and Mitchison[8] described a new slide culture technique that was rapid, simple and safe, but it required the use of fluorescent microscope. We have modified the above technique to obtain similar results with the use of bright field microscope.

Material and Methods

(a) Sputum specimens were collected in sterile wide mouth bottles from untreated smear posi tive patients of pulmonary tuberculosis. Ten to twelve sterile glass beads were added to each specimen which was then stirred for 5 minutes to homogenize the material.

(b) Glass slides of thin glass were used. Each slide was split longitudinally with a glass knife. Each split slide was marked with patient's num ber and drug code with a glass marking pencil.

(c) *Selective culture medium :* Outdated, but not more than 4 weeks old, citrated blood obtained from a Blood Bank was used. An equal amount of sterile deionized water was added and stirred till hemolysis was complete. The medium was then made selective by adding Polymixin B 200 units/ml, carbenicillin 100 mg/L, trimethoprim lOmg/L and amphotericin B lOmg/L.

The medium was poured into sterile glass bottles. No antituberculosis drug was added to bottle No. 1. To bottles No. 2, 3 and 4, Isoniazid was added to make drug concentrations of 0.1 mg/L, 0.2 mg/L and 0.4 mg/L respectively. To bottles No. 5, 6 and 7, was added Streptomycin to make concentrations of 1.0 mg/L, 2.0 mg/L and 4.0 mg/L respectively. To bottles No. 8, 9 and 10, Rifampicin was added to make concentrations of 0.2 mg/L, 0.4 mg/L and 0.8 mg/L respectively and to bottles No. 11, 12, and 13, Ethambutol to make 0.5 mg/L, 1.0 mg/L and 2.0 mg/L respectively.

Ten ml each of drug free and drug containing

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Correspondence : Dr. P.R. Gupta, A-2 Khetri House, Jaipur- 302016

media were poured aseptically into sterile 28 ml McCartney bottles, when required.

\ *Procedure*

Each treated sputum specimen was spread fairly thickly over the lower 2/3rd of each split slide using a wire loop; 16 such slides were made out of each specimen. These slides were then allowed to dry before being placed into drug free and drug containmg"media. The remaining slides were kept as control.

The slide cultures were incubated for 7 days at 37°C.* The slides were then removed with a pair of forceps and dipped in water in discard bins for 5 minutes to remove excess of blood. The back of each slide was wiped clean with cotton before being placed in a slide rack, air dried and then placed in oven for 30 minutes at 80°C. These slides were then stained by Z.N. technique and examined under bright field microscope for presence of micro colonies.

The microcolonies were graded as under:

0- No multiplication of acid fast bacilli seen as compared with an unincubated control smear.

1 + Small clumps of upto 4 bacilli.

2+ Large clumps of bacilli but no cord formation.

3 + Micro-colonies with some cord formation. 4+ Large micro-colonies with good cord formation.

For every 10 sputum specimens examined, one control slide culture was also put up using $H_{37}R_v$ strain of *M. tuberculosis obtained through a seed lot system.*

Specimens showing growth of grade 3 + *or 4+ only were considered for analysis of the sensitivity pattern.*

Indirect drug sensitivity tests were done on all the 100 sputum specimens on slopes of Lowen-stein Jensen medium, as per the method of Can-etti et at,[9] for comparison.

(e) Drug concentrations and definition of resistance

The minimal drug concentrations of various drugs used which prevented a growth of at least 1 grade less than that on drug free media of $H_{37}R_V$ strain were considered as the critical drug concentrations.

Resistance was assumed if a specimen showed equivalent or 1 grade less growth on the critical drug concentration as compared with the drug free media, i.e., if drug free media showed a growth of 3 +, a 2 + or more growth on the critical drug concentration of drug media was considered as drug resistant.

Results

A 4+ growth was obtained in all the 10 control $H_{37}R_v$ cultures on drug free media. The critical concentrations of antituberculosis drugs which prevented a 3 + or more of growth on slide culture were as under:

Streptomycin Isoniazid	2.0mg/L
Ethambutol Rifampicin	0.2mg/L
	l.Omg/L
	0.8mg/L

These drug concentrations were, therefore, taken as cut off drug concentrations. Since these drug concentrations were similar to those used by Dickinson and Mitchison[8], resistance was assumed if the drug culture showed growth as under:

Drug	Grade[4]
Streptomycin	3+
Isoniazid	2+
Ethambutol	3+
Rifampicin	2+

The growth pattern of the 100 sputum specimens on drug free slide cultures was as under:

Grade of growth	No. specimens
Total	100
3+	39
0	2

Compared to the above results, 97 specimens were positive on LJ Media. The remaining 3

The slide culture bottles were opened to fresh air every day during this period.

[+]For sputa samples showing grade III growth on drug free media, a growth of 1 grade less than this on drug media was considered as drug resistance.

Table 1. *Results of drug sensitivity testing with slide culture and indirect sensitivity techniques*

Drug (s)	No. showing resistance	
	Slide Culture	Indirect sensitivity test
Streptomycin	5	6
Isoniazid	3	3
Rifampicin	1	2
Ethambutol	2	2
Streptomycin + Isoniazid	1	1
Streptomycin + Rifampicin	1	1
Streptomycin + Ethambutol	2	1
Isoniazid + Ethambutol	–	–
Izoniazid + Rifampicin	2	1
Ethambutol + Rifampicin	–	–
Total	17	17

samples showed contamination.

As 83 specimens showed 3+ or more growth on slide culture, these were considered eligible for further analysis. The corresponding indirect sensitivity tests were also considered for comparison.

The results by slide culture technique and indirect sensitivity testing and the correlation between the two tests are shown in Tables 1 and 2 respectively.

Discussion

Although the slide culture technique is recommended to be used for the management of treatment failure patients, yet sputum specimens for the present study were obtained only from untreated patients of pulmonary tuberculosis for a scientific comparison.

In all 83 out of the total 100 sputum specimens tested showed a growth of 3+ to 4+ on slide cul-

Table 2. *Correlation of drug sensitivity by slide culture and indirect testing techniques*

Slide culture sensitivity results	Indirect Sensitivity Results											
	Sensitive to all drugs	S	H	E	R	S+H	S+E	S+R	H+E	H+R	E+R	Total
Sensitive to all drugs	64	1	-	-	1	-	-	-	-	-	-	66
Resistant to SM	-	5	-	-	-	-	-	-	-	-	-	5
Resistant to INH	-	-	3	-	-	-	-	-	-	-	-	3
Resistant to RIF	-	-	-	-	1	-	-	-	-	-	-	1
Resistant to ETH	-	-	-	2	-	-	-	-	-	-	-	2
Resistant to S+H	-	-	-	-	-	1	-	-	-	-	-	1
Resistant to S+R	-	-	-	-	-	-	-	1	-	-	-	1
Resistant to S+E	1	-	-	-	-	-	1	-	-	-	-	2
Resistant to H+E	-	-	-	-	-	-	-	-	-	-	-	-
Resistant to H+R	1	-	-	-	-	-	-	-	-	1	-	2
Resistant to E+R	-	-	-	-	-	-	-	-	-	-	-	-
Total	66	6	3	2	2	1	1	1	-	1	-	83

ture. A majority of the remaining 17 sputa had low positively on smear also, i.e., 13 were grade 1+ and 4 were grade 2 + . Since the practical use of this technique will be in the treatment failure patients with highly positive sputa, a higher percentage positivity in grade 3 + or 4 + on slide culture can safely be assumed.

Only the 83 cultures which showed a 3 + or 4 + growth were further analysed: 66 out of them showed bacillary growth sensitive to all the drugs tested. The remaining 17 showed drug resistance to 1 or more of the drugs. Compared with the indirect sensitivity test results, there was 95.2% agreement between the two methods. An agreement at the 90-95 per cent level between the two methods is considered as good[3]-

The above results were obtained by a technique which is simple, safe, rapid and cheap and is, therefore, considered most suitable for countries where the need for the rapid sensitivity testing to manage a large number of sick, smear positive patients on long unsuccessful chemotherapy is to be met.

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